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(54) Title: RECEPTOR, THE USE THEREOF, AND MOUSE ANTIBODIES

(54) Bezeichnung: REZEPTOR, DESSEN VERWENDUNG SOWIE MAUSANTIKÖRPER

(57) Abstract: The invention relates to a receptor located on the surface membrane of highly proliferative cells, particularly of the gastric carcinoma, which is composed of glycoproteins. At least one determinant of the glycoprotein corresponds with one of the CFR-1 protein, and the human antibody 103/51 and/or the murine antibody 58/47-69 (IgM) specifically binds to the glycoprotein.

(57) Zusammenfassung: Rezeptor auf der Oberflächenmembran von stark proliferierenden Zellen insbesondere des Magenkarzinoms, der aus Glykoproteinen aufgebaut ist, wobei wenigstens eine Determinante des Glykoproteins mit einer des CFR-1 Proteins übereinstimmt und der humane Antikörper 103/51 und/oder der murine Antikörper 58/47-69 (IgM) am Glykoprotein spezifisch bindet.

Receptor, The Use Thereof, and Mouse Antibodies

The present invention relates to a receptor found on the surface of rapidly proliferating cells, particularly gastric carcinoma cells, its use, and the structure of a mouse antibody which binds specifically thereto.

Using monoclonal antibodies generated from hybridomas for clinical and scientific assays is widely known. The administration of human monoclonal antibodies produced from B-cell hybridomas is promising for the treatment of tumors, viral and microbial infections, B-cell immunodeficiencies with reduced antibody production, and other impairments of the immune system.

Gastric carcinoma is one of the most frequently occurring types of cancer worldwide. According to Lauren, "The two histological main types of gastric carcinoma," Acta Path. Microbiol. Scand. 64:331-49, it is histologically divided into diffuse adenocarcinoma and intestinal adenocarcinoma. Intestinal gastric carcinomas are often accompanied by chronic type B gastritis and particularly by intestinal metaplasias, which are considered to be precursors of dysplastic changes and of gastric carcinomas. Differences between these two types are also shown in that patients having carcinomas of the diffuse type often belong to blood group A, from which the influence of genetic factors on the cancer risk may be concluded, while environmental factors, e.g., a *Helicobacter pylori* infection, is possibly significant for the occurrence of carcinomas of the intestinal type. A reduced frequency of gastric adenocarcinoma has been established in the West, but it is now increasingly occurring in the East.

The development of stomach cancer is a multi-step and multi-factor process (Correa, 1992, Cancer Res. 52:6735-6740). Although little is known about molecular mechanisms, factors such as high salt intake, alcohol, nitrosamines, and infection with the bacterium *Helicobacter pylori* (*H. pylori*) are clearly proven to be involved in the initiation of stomach carcinogenesis. Due to a strong correlation between *H. pylori* infection and the occurrence of gastritis, dysplasia, and development of gastric cancer, the bacterium has been classified as a class I carcinogen by the WHO. *H. pylori*

predominantly expressed on lymphocytes, but are also found on various other cell types, wherefore they are not suitable for cancer therapy. In particular, ligands and antibodies for these receptors have led to liver damage in *in vivo* tests. Therefore, tumor-specific receptors having apoptotic function are especially important.

In recent publications, we described that the human antibody 103/51, which was isolated from a stomach cancer patients with diffuse-type adenocarcinoma, cross-reacts with *H. pylori* and stomach cancer cells (Vollmers *et al.*, 1994, Cancer 74:1525-1532). In all assays, the known gastric adenocarcinoma cell line 23132 was used, which is deposited under No. ACC201 at the DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Mascheronder Weg 1b, 38124 Braunschweig. In low doses, the antibodies have a mitotic effect on stomach cancer cells *in vitro*, in which they bind on a 130 kD membrane receptor (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235). The antibody has some mitotic effect on stomach carcinoma cells *in vitro* by binding to a 130 kD membrane receptor (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235). Sequencing of the antibody variable gene regions identified the antibody 103/51 as an autoreactive antibody. Immunohistochemistry studies show that the antibody reacts strongly with stomach cancer cells and with glandular stomach cells.

The cellular receptor of monoclonal antibody 103/51 was previously unknown. In the course of the experiments leading to the present invention, we were able to identify this cellular receptor. However, this identification proved to be difficult. On one hand, the monoclonal antibody 103/51 reacts with its receptor during Western blot analysis only under very specific stringency conditions. On the other hand, non-specific reactions are found with an array of further proteins, caused by denaturing artifacts.

Sequencing analyses have shown that the receptor corresponds to the CFR-1 protein, but is not identical to this protein. Furthermore, glycoprotein compounds which have one or more determinants (ligands) corresponding to those of the known CFR-1 are thus claimed. In particular, a homology is required which is to be defined according to this application as a correspondence of at least 80% in the primary amino acid

sequences. The receptor is therefore an isoform to CFR-1. In addition, specific binding to either the human antibody 103/51 and/or the murine antibody 58/47-69 is required.

It is of special interest if the specific binding site on the glycoprotein is a carbohydrate residue, i.e., a sugar residue.

In a special embodiment, the CFR-1 protein has an amino acid sequence according to Appendix S, cell line 23132 as a determinant.

The cellular receptor of the antibody 103/51 is an isoform of the protein CFR-1, specific for tumor cells, particularly for gastric carcinoma cells, which does not occur in normal tissue. The specific receptor properties of this isoform are based on a special glycostructure linked to the protein backbone via an N-linkage. The tumor-specific receptor may be used in a screening method for identifying specific binding partners. According to the present invention, specific binding partners on the receptor are those compounds which bind selectively to a tumor-specific glycostructure of CFR-1 and preferably have the ability to induce apoptosis. These specific binding partners may be used for the production of therapeutic agents for the treatment of tumors and for the production of diagnostic agents.

The protein compound was characterized as an isoform of CFR-1 through purification, sequencing, and transfection. The specificity for the antigen 103/51 was confirmed by producing murine antibodies from purified molecules having identical reactions and functions, through immunohistochemical staining, and an MTT assay of two CFR-1 negative cell lines. The isoform of the CFR-1 molecule, which was detected by both the human and the murine antibodies, is localized in the cell membranes of the epithelial cells and has an expression pattern which differs from that previously described for CFR-1 (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609).

CFR-1, which was isolated as a high-affinity FGF-binding protein from chicken fibroblasts (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609), binds to a number of

FGFs and may have a role in the regulation of cellular proliferation. In Chinese hamster ovary cells (CHO), CFR-1 was found to be expressed only in the Golgi apparatus (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609), but it can also be secreted in a mutated form (Zuber *et al.*, 1997, J. Cell Physiol. 170:217-227). Depending on the organism, two detected variants of CFR-1, ESL-1, and MG-160 share sequence homologies between 80% and 95% (Burrus *et al.*, 1992, Mol. Cell. Biol. 12:5600-5609; Stieber *et al.*, 1995, Exp. Cell Res. 219:562-570; Steegmaier *et al.*, 1995, Nature 373:615-620; Mourelatos *et al.*, 1996, DNA Cell Biol. 15:1121-1128) and do not appear to share any sequence homologies to other known proteins. Function and cellular distribution of CFR-1 and the homologues is relatively unknown and contradictory. It has been shown that MG-160, which is a medial Golgi sialoglycoprotein and was purified from rat brains, plays a role in intracellular FGF trafficking (Zuber *et al.*, 1997, J. Cell Physiol. 170:217-227).

Recent findings have shown that the localization of this protein is not restricted to the Golgi apparatus. However, if truncated at the c-terminus, the protein can be localized to the plasma membrane and filopodia (Gonatas *et al.*, 1998, J. Cell Sci. 111:249-260). This is consistent with the finding that the third homologue, ESL-1, which was isolated from mouse neutrophilic progenitor cells (32Dcl3), is located in the Golgi apparatus as well on the cell surface of the microvilli (Steegmaier *et al.*, 1997, J. Cell Sci. 110:687-694, Gonatas *et al.*, 1998, J.Cell Sci. 111:249-260). ESL-1 was identified as ligand for E-selectin in neutrophils with an approximate molecular mass of 150 kD. Immunoprecipitation with anti ESL-1 antibodies showed that a non-defined isoform of this protein could be precipitated from various cells, including some cancerous cell lines (Steegmaier *et al.*, 1995, Nature 373:615-620).

Because of the predominantly membranous distribution of CFR-1 in cancerous cells, we conclude that the described receptor is an isoform of CFR-1. A variable cellular distribution of CFR-1 and its homolog is probably responsible for the results cited and is a known phenomenon for other proteins (Smalheiser, 1996, Mol. Biol. Cell 7:1003-1014). An altered distribution might be caused by a different glycosylation pattern in malignant cells, which may lead to a transport to the plasma membrane.

The tissue distribution shows that the CFR-1 molecule is correlated with cellular activation and proliferation demonstrated by staining with antibody Ki67 (Ramires *et al.*, 1997, J. Pathol. 182:62-67). Normal stomach mucosa does not express this receptor in a measurable amount, but *H. pylori* infiltrated epithelia and dysplastic epithelia have this antigen. Both tissues proliferate and may be precursors for gastric carcinoma.

To understand the high effectiveness, it is important to note that in contrast to the structure of CFR-1, which is found in healthy cells, the characterized isoform is not found on healthy cells, but exclusively on rapidly proliferating cells, i.e., cells which rapidly divide, such as the tumor cells found in the growth and corresponding precursor stages. The function of the receptor is essentially based on it being used as an energy receptor for nutrition intake of the cells and having a dominant share particularly in frequently dividing cells, such as carcinoma cells. It is to be expressly noted that this receptor will have applications not only in gastric carcinomas, but rather also for all epithelial tumors which have essentially the same reaction mechanisms. Besides gastric tumors, the existence of these receptors was proven in cancerous tissue of the following tumors: esophagus, stomach, intestines, rectum, liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, cardiac, Barrett's, ovary, and/or uterus. The antibodies effective on the tumors, which bind to the receptor according to the present invention, therefore have a targeted activity on the cancerous (and not the healthy) cells.

The glycoproteins of the receptor structure were able to be identified via their molecular mass of approximately 130 kD, the molecular mass able to be determined using a known method, for example, using gel electrophoresis. The term "approximately" is based on the fact, recognizable to one skilled in the art, that these types of size determinations are not exact in any way, but rather changes or variations of the methods of the molecular size determination lead to variations in the measurement values.

The most significant field of application of the receptor is diagnosis and therapy. For prophylactic application, the receptor is administered to the patients in pharmaceutical doses, with the goal of stimulating antibodies, so that vaccination may be achieved with the aid of the receptor. The antibodies are responsible for removing any tumor cells which arise.

However, the administration of the receptor if tumor cells are already present is also a possibility for medication. The administered receptors reinforce and amplify antibody formation and therefore are responsible for elevated apoptosis of the tumor cells or for a complement-mediated lysis. The cells "starve," since blocking of the receptor leads to growth arrest.

The assays up to this point have shown that the receptor has been proven particularly suitable for treating the following tumor precursors. In regard to illnesses of the stomach, the receptor is suitable for treating dysplasia of the gastric mucosa and/or intestinal metaplasia of the stomach and/or for treating inflammation of the gastric mucosa which is associated with the bacteria *Helicobacter pylori* and for treating tubular and tubulovillous adenomas of the stomach. Application is also indicated for the following diseases of the colon, specifically tubular adenoma of the colon, villous adenoma of the colon, and dysplasia in ulcerative colitis. The receptor is also suitable for Barrett's dysplasia and Barrett's metaplasia of the esophagus. The receptor is also suitable for treating the following diseases of the cervix: cervical intraepithelial neoplasia I, cervical intraepithelial neoplasia II, and cervical intraepithelial neoplasia III.

Finally, the receptor described above is also suitable for administration with squamous epithelial metaplasia and squamous epithelial dysplasia of the bronchus.

Due to the operative mechanisms described above, the receptor is suitable in principle for treating tumors of the esophagus, the stomach, intestine, the rectum, the liver, gallbladder, pancreas, lungs, bronchi, breast, cervix, prostate, cardiac, Barrett's, ovary, and/or uterus.

The application of the receptor for diagnosis purposes uses the ability of the antibody to bind to this receptor due to the specific antigen/antibody interaction. In this way, evidence for the existence, the localization, and/or the quantity of the corresponding antibodies may be derived from the ability to bind to the receptor. With the same reaction mechanisms, the binding ability may be used to detect the receptor.

Particularly if the antibodies are tumor antibodies, they may be used to detect the existence of tumors. In particular, it is possible to use the receptor as a tumor marker.

In a refinement, the receptor may be used to produce an antitumor agent, in which compounds that are potentially effective against tumors are assayed for their ability to specifically bind to the receptor and upon a positive result, i.e., upon the occurrence of binding, this compound is used for the pharmaceutical application. Of course, appropriate formulation and the addition of typical additives is necessary, as usual, for producing a pharmaceutical which reaches the market.

It remains to be expressly stated that not only human antibodies come into consideration for the production of antitumor medications with the aid of the receptor as described above, but rather also mouse antibodies and/or humanized antibodies of any arbitrary species. This is also true for antibody fragments such as Fab and $F(ab)_2$ and/or Fab' fragments, as are obtained through proteolytic cleavage of antibodies. These also include single strand antibodies and/or tetrameric and/or dimeric antibody forms and/or bispecific antibodies.

Furthermore, it is known that human tumor antigens which are immunogenic in mice are used for generating monoclonal mouse antibodies and are capable of specifically recognizing the human antigen and therefore are suitable for being used therapeutically in humans.

The object of the present invention is the establishment of the receptor structure and its use. However, the repeated injection of "foreign" antibodies and/or mouse antibodies into humans is problematic as it leads both to disadvantageous hypersensitivity reactions and to elevated clearance rate of the circulating antibodies, so that the antibodies do not reach their target location.

For these reasons, reexamination of the therapeutic suitability of mouse antibodies is required. Nonetheless, the suitability in connection with diagnostic methods is unrestricted. The possibility of deriving humanized mouse antibodies and using them for therapeutic purposes also exists. It is also decisive that not only existing tumors, but also pre-cancerous structures may be characterized with the aid of these diagnostic methods.

In addition to the receptor described above, protection is also claimed for a mouse antibody which binds specifically thereto, whose structure is defined by Appendices A and B. The regions identical for all antibodies were not reproduced; those regions characteristic for the individual antibody were claimed and shown.

As a result, the receptor whose structure is described, which should be designated as an isoform of CFR-1, enables the therapy and diagnosis not only of tumors, but also of pre-cancerous structures. In addition, the structure of a mouse antibody which binds specifically thereto is described.

Material and Methods

Cell culture and antibody purification

For all assays, the established stomach adenocarcinoma cell line 23132 (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235) was used. Cells were grown to 80 % confluency in RPMI-1640 (PAA, Vienna, Austria) supplemented with 10% FCS and penicillin/streptomycin (1% for both). For the assays described, cells were detached with trypsin/EDTA and washed twice with phosphate buffered saline (PBS) before

use. The human hybridoma cell line 103/51 was produced and grown as described (Vollmers *et al.*, 1994, Cancer 74:1525-1532). Purification of the IgM antibodies was performed as described elsewhere (Vollmers *et al.*, 1998, Oncol. Rep. 5:549-552).

Preparation of membrane extracts

Isolation of membrane proteins from tumor cells was performed as described by Hensel *et al.* (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235), using cell line 23132. In short, confluent tumor cells were washed twice with PBS, harvested with a cellscraper and centrifuged, and resuspended in hypotonic buffer (20 mM HEPES, 3 mM KCl, 3 mM $MgCl_2$). After 15 min incubation on ice, followed by sonification for 5 min, the nuclei were pelleted by centrifugation at 10,000g for 10 min. The supernatant was centrifuged for 30 min at 100,000g in a swing-out rotor to pellet membranes. After washing the pellet with hypotonic buffer, it was resuspended in membrane lysis buffer (50 mM HEPES pH 7.4, 0.1 mM EDTA, 10% glycerol, and 1% Triton X-100). A protease inhibitor (Boehringer, Mannheim, Germany) was added to all solutions.

Western blotting

10% reducing SDS-PAGE gels and Western blotting of proteins were performed using standard protocols as described elsewhere (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235). In short, blotted nitrocellulose membranes were blocked with PBS containing 2% low fat milk powder, followed by 1 h incubation with 10 µg/ml purified antibody 103/51. The secondary antibody (peroxidase-coupled rabbit anti-human IgM antibody (Dianova, Hamburg, Germany)) was detected with the SuperSignal chemiluminescence kit from Pierce (KMF, St. Augustin, Germany). After three washings with PBS + 0.05% Tween-20, the second antibody (peroxidase-coupled rabbit antihuman IgM antibody (Dianova, Hamburg, Germany)) was incubated. The reaction was detected with the aid of the SuperSignal chemiluminescence kit from Pierce (KMF, St. Augustin, Germany).

Purification of the antigen 103/51

The purification of the antigens were performed by column chromatography using a Pharmacia (Freiburg, Germany) FPLC unit. For size exclusion chromatography, a Pharmacia Superdex 200 column (XK16/60) was loaded with 5 mg membrane

preparation and run with buffer A (100 mM Tris/Cl, pH 7.5, 2 mM EDTA, 40 mM NaCl, 1% Triton X-100). Then, the eluate was fractionated and examined in Western blot analysis for reaction with antibody 103/51. Positive fractions were loaded on a MonoQ (5/5) column using buffer A. The bound proteins were eluated with a linear gradient using buffer B (100 mM Tris/Cl, pH 7.5, 1 M NaCl, 2 mM EDTA, 1 M NaCl, 1% Triton X-100), fractionised and examined in Coomassie-stained SDS-PAGE and Western blot analysis. Positive bands were cut out from gel and sequenced or used for immunization of mice.

MALDI peptide mapping

The band of interest was excised and cut into small pieces of about 1 mm x 1 mm. Gel pieces were washed, reduced with DTT, S-alkylated with iodoacetamide, and in-gel digested with trypsin (unmodified, sequencing grade, Boehringer) as described elsewhere (Shevchenko *et al.*, 1996, Anal.Chem. 68:850-858). After 3 h of digestion at 37°C, 0.3 µl of the digest solution was removed and subjected to MALDI peptide mass mapping on a Bruker Reflex MALDI-TOF equipped with delayed extraction (Bruker-Franzen, Bremen, Germany). The thin film technique was adopted for sample preparation (Jensen *et al.*, 1996, Rapid.Commun.Mass.Spectrom. 10:1371-1378). The tryptic peptide masses were used to search a non-redundant protein sequence database by the PeptideSearch software program developed in-house.

Cloning of CFR-1 anti-sense vector and transfection

RNA isolation, cDNA synthesis, and PCR were performed as described (Hensel *et al.*, 1999, Int.J.Cancer 81:229-235). In short, for PCR for amplification of a 897 bp fragment ranging from basepairs 802 to 1699, the following primers were used: CFR-For 5' GCTTGGAGAAAGGCCTGGTGAA 3', CFR-Rev 5' TGGCACTTGCGGTACAGGACAG 3'. Amplification was performed using the following cycle profile: 95°C, 2 min, followed by 35 cycles of 94°C, 30 sec; 60°C, 30 sec; 72°C, 60 sec, and a final extension of 72°C for 4 min. Cloning into the pCR-Script Amp SK (+) vector and DNA sequencing were performed as described before (Hensel *et al.*, 1999, Int. J. Cancer 81:229-235). The insert was subcloned into the pHook-2 vector (Invitrogen, Leek, Netherlands), and cloning was controlled again by sequencing.

Transfection of cell line 23132 with pHOOK2-antiCFR-1 was accomplished with PrimeFector reagent (PQLab, Erlangen, Germany) according to supplier's manual. In short, plasmid DNA was diluted to 10 µg/ml and the prime factor reagent was added in a 1:10 ratio to a serum-free growth medium. Diluted plasmid DNA (450 µl), diluted Primefector reagent (90 µl), and serumfree medium (460 µl) were mixed and incubated at RT. 60-milliliter cell culture plates (70% confluent) were washed two times with serumfree medium, and then the PrimeFector/DNA mixture was added dropwise. Cells were incubated 18 h at 37°C and 7% CO_2, then serumfree growth medium was replaced with growth medium containing 10% FCS, and cells were incubated another 24 h before studying CFR-1 expression.

Flow cytometry

The cell line 23132 was detached from culture plates by trypsin /EDTA 48 h after transfection, washed and subsequently incubated on ice with antibody 103/51 and human) isotype-matched control antibody (Chromopure human IgM) for 15 minutes, followed by incubation with a FITC-labeled rabbit anti-human IgM antibody (Dianova) for 15 minutes on ice. Antibodies were optimally diluted in PBS containing 0.01% sodiumazide. Cells were analyzed by flow cytometry (FACScan; Becton Dickinson, USA).

Glycosidase assays

Detached and washed cells were resuspended in RPMI-1640 containing 10% FCS and incubated for 1 h on ice, then counted, and cytospins were prepared. After air-drying, cytospin preparations were acetone-fixed (10 min), washed, and incubated with 20 µU/ml O-glycosidase or 5 mU/ml N-glycosidase (Boehringer) for 4 h at 37°C. Then, slides were washed and immunohistochemically stained.

For deglycosylation of membranous proteins, membrane extracts were incubated for 16 h at 37°C with 1 mU/ml N-glycosidase diluted in deglycosylation buffer (50 mM PO_4-Buffer, pH 7.4). As a control, extracts were incubated with deglycosylation buffer alone. Then, extracts were separated by SDS-PAGE and Western blots were performed as described above.

Production of murine monoclonal antibodies

BALB/c mice were immunized two times within 17 days with 5 µg purified antigen of antibody 103/51, and killed 4 days after the second immunization. Spleens were disrupted mechanically and fused with 1 x 10^7 NS0 cells as described earlier (Vollmers *et al.*, 1985, Cell 40:547-557). Antibody-producing hybridomas were tested through immunohistochemical staining and reaction in Western blot analysis. Clone 58/47-69 with positive reactivity was used for further experiments.

Immunohistochemical staining of paraffin sections

Paraffin-embedded human gastric mucosa and tumor were sectioned (5 µm), deparaffinized, and blocked with BSA (15 mg/ ml) diluted in PBS for 30 min. The sections were incubated with supernatant of hybridoma 103/51, or 58/47-69, Ki67 (Loxo, Dossenhein, Germany) or mouse anti-cytokeratin 8 antibody diluted 1:15 with BSA/PBS (Dako, Hamburg, Germany) for 2 h in a humidified incubator. Then they were washed three times with Tris/NaCl, followed by incubation with peroxidase-labeled rabbit anti-human or rabbit anti-mouse conjugate (Dako) diluted 1:50 in PBS containing rabbit serum (for antibody 103/51) or in PBS containing human AB plasma (for antibody 58/47-69 and anti-cytokeratin). After washing three times with Tris/NaCl and incubation in PBS for 10 min staining was performed with diaminobenzidine (0.05%)-hydrogen peroxide (0.02%) for 10 min at RT. The reaction was stopped under running tap water, and sections were counterstained with hematoxylin.

Immunohistochemical staining of living and acetone-fixed cells

For living cell staining, cells were detached, washed and diluted to $1x10^6$ cells/ml. 1 ml of cell suspension was centrifuged at 1,500g for 5 min. Antibody diluted to 40 µg/ml with complete RPMI was added to a final volume of 1 ml and incubated for 90 min on ice. Then cells were pelleted at 1,500g for 5 min and resuspended with 500 µl RPMI. With 200 µl of the cell suspension, cytospin preparations were prepared and air-dried for 30 min. Cells were fixed in acetone for 30 min and washed with

Tris/NaCl three times. HRP-coupled rabbit anti human IgM (DAKO) was diluted 1 : 50 in PBS/BSA (0,1 %) and incubated for 30 min at RT. After three washings, staining was performed as mentioned above.

For staining of acetone-fixed cells, cytospins were prepared, air-dried at RT and fixed in acetone as described above. Then, cytospins were blocked for 15 min with PBS/BSA (0.1 %) and incubated for 30 min with 10 µg/ml primary antibodies followed by three washings. Incubation with secondary antibody and staining was performed as described above.

MTT-proliferation assay

The MTT-assay with the established cell line 23132 was performed as described (Vollmers *et al.*, 1994, Cancer 74:1525-1532). In short, trypsinized cells were diluted to $1x10^6$ cells/ml in complete growth medium, and 50 µl of cell suspension was added to each well of a 96-well plate. Then 50 µl of the antibodies, diluted to the indicated concentrations with complete growth medium, were added to the wells, and plates were incubated for one or two days at 37°C in a humidified incubator. For measurement, 50 µl of MTT (3(4,5 dimethylthiazol)-2,5 diphenyltetrazolium bromide) solution (5 mg/ml) were added to each well, and plates were incubated for 30 min. After incubation, plates were centrifuged at 800g for 5 min, MTT solution was removed, the stained cell pellet was dissolved in 150 µl dimethylsulphoxide, and absorption was measured at wavelengths of 540 nm and 690 nm.

Methods of determining the sequence of CFR-1

RNA was prepared for the cDNA synthesis with the aid of the RNeasy kit from Quiagen. For preparation, $1x10^6$ cells were washed twice using ice cold PBS and pelletized at 1000 x g for 5 minutes and the RNA was prepared in accordance with the manufacturer description. 5 µg RNA (1-5 µl solution) was mixed with 1 µl oligo-dT_{15} (1 µg/µl) and 2 µl random primer (40 µM) and filled up to a total volume of 8 µl using H_2O. The RNA was denatured for 10 minutes at 65°C and the sample was subsequently cooled on ice. 17 µl Mastermix, consisting of 5.2 µl DEPC-H_2O, 5 µl 5x

reverse transcriptase buffer, 2.5 µl dNTPs (per 10 mM), 2.5 µl DTT (250 mM), 0.8 µl RNasin (400 U), and 1 µl M-MLV reverse transcriptase (200 U), was then pipetted thereto. The synthesis of the cDNA was performed for 70 minutes at 37°C and was subsequently terminated by heating to 95°C for 5 minutes. 1-5 µl of the cDNA was mixed with the PCR Mastermix and filled up to 25 µl total volume using H_2O. The PCR Mastermix consisted of 2.5 µl 10x Taq-polymerase buffer, 0.5 µl 10 mM NTPs, 1.5-2 µl 25 mM $MgCl_2$, 0.5 µl each 20 pM 3' and 5' primer, and 0.2 µl Taq polymerase (1 U). The amplification conditions for the various PCR products are listed in the following table.

Overview of the PCR program used for amplifying the various cDNAs

Product	Annealing in [°C]	$MgCl_2$ [mM]	Extension time [seconds]	Cycles	Product size [bp]
Fragment 1	55	1.75	45	40	691
Fragment 2	60	1.5	45	40	898
CFR Fragment 3	55	2.0	45	40	739
Fragment 4	55	2.0	45	40	941
Fragment 5	55	2.0	45	40	750

Primer sequences

Sequences for the oligonucleotides used for the PCR

CFR

CFR-For 1	5'	OGC AGC TTC AGC AGC AAC AGC A	3'
CFR-Rev 1	5'	CAG CTC AGC CAC CCG GAG AAT G	3'
CFR-For 2	5'	GCT TGG AGA AAG GCC TGG TGA A	3'
CFR-Rev 2	5'	TGG CAC TTG CGG TAC AGG ACA G	3'
CFR-For 3	5'	GAA CAC CGT CTC TTA GAG CTG C	3'
CFR-Rev 3	5'	GCT TCC TGC AGA GTG TCA TTG C	3'
CFR-For 4	5'	GGA GGA CGT GTT GAA GCT TTG C	3'
CFR-Rev 4	5'	CCA GGG CAC AAG CAG TAT GAA G	3'

CFR-For 5	5′	CAA CAG CAG ACA GGT CAG GTG G	3′
CFR-Rev 5	5′	CCG GAA GTT CTG TTG GTA TGA G	3′

The sequencing was performed using a sequencer from the firm Applied Biosystems. The following oligos were used for the sequencing of cloned PCR products:

T_3	5′	ATT TAA CCC TCA CTA AAG GG	3′
T_7	5′	GTA ATA CGA CTC ACT ATA GGG C	3′

3 µl plasmid DNA was mixed with 1 µl primer (3.2 pM), 11 µl H_2O, and 5 µl reaction mixture of the AbiPrism Sequencing Kit and incubated in the thermocycler for 25 cycles using the following parameters:

Denaturing	Annealing	Extension
95°C, 30 seconds	52°C, 15 seconds	60°C, 4 min.

To remove oligos and dNTPs, the reaction mixture was purified via a Sephadex G-50 column. For this purpose, a 100 µl pipette tip was loaded up to the upper edge with column material and centrifuged for 3 minutes at 2000 x g. Subsequently the sample was applied and the small column was centrifuged again. The DNA was then precipitated by 2 µl Na acetate (pH 5.2) and 50 µl 100% ethanol and pelletized by centrifuging at 13,000 x g for 15 minutes. After drying, the DNA was received in 3 µl formamide/25 mM EDTA (5:1) and analyzed in the sequencer.

Analysis of the Sequencings

At least five clones were sequenced from all clonings. In order to remove errors which arose during the amplification using the Taq-polymerase and/or the sequencing, the sequences of the cloned PCR fragments were compared with one another with the aid of the DNAsis for Windows software and a consensus sequence of all clones was established from both read directions. By rewriting the DNA sequences into amino acid sequences, the number of silent mutations and amino acid

substitution mutations were determined. The sequences for MG160 and CFR were drawn from the NCBI databank and compared to sequencings of the PCR products using the DNAsis for Windows program.

Figures and Tables

Figures and Tables

Fig. 1: Identification of the antigen of antibody 103/51

a) Protein purification of the antigen from membrane extracts of stomach carcinoma cell line 23132. Membrane fractions were processed by chromatographic procedures and whole membrane fraction (lane 2), or purified proteins (lane 3) were stained with Coomassie (lane 1: 10 kDa ladder). Western blot analysis with antibody 103/51 on membrane fractions of cell line 23132 showed one reaction with a protein with a molecular mass of approximately 130 kD (lane 4). Specificity of processed membrane extracts was controlled by Western blotting with 103/51 (lane 5). The protein band indicated by the arrow was excised from a preparative gel and used for MALDI mass mapping and immunization of mice.

b) Identification of the 130 kDa gel-separated protein by high resolution MALDI peptide mass mapping. Peaks labeled with '*' match the calculated masses of tryptic peptides of U28811 human cysteine-rich fibroblast growth factor receptor (CFR-1) with a mass accuracy better than 50 ppm. Peaks labeled with 'T' correspond to trypsin autolysis products. The inset shows the mass resolution ($m/\Delta m = 9000$) of the peak at m/z 1707.818.

Fig. 2: Effect of CFR-1 antisense transfection on antibody 103/51 staining and live cell staining (Magnification 200x)

a) Cell line 23132 transiently transfected with control vector and acetone fixation shows intensive staining with antibody 103/51.

b) Reduced staining is visible in cells transiently transfected with CFR-1 antisense vector.

c) To reduce background staining in immunohistochemical staining, live cell staining was performed with cell line 23132. A clear membrane staining is visible.

d) Control live cell staining (only secondary antibody) on cell line 23132.

e) Negative live cell staining on cell line Colo-699 with antibody 103/51 indicates that this cell line is negative for expression of CFR-1.

f) Control live cell staining (only secondary antibody) on cell line Colo-699.

g) Flow cytometry of cell line 23132 with antibodies Chromopure human IgM (grey) and 103/51

h) Analysis of cells transfected with control vector pHOOK-2 with flow cytometry 48 h after transfection.

i) Cells transfected with CFR-1 antisense vector shows a clear decrease in binding of antibody 103/51

Fig. 3: Effect of deglycosylation on staining with antibody 103/51

a) Cells (23132) incubated with deglycosylation buffer and acetone-fixed show intense staining with antibody 103/51.

b) Cells (23132) treated with N-glycosidase followed by acetone fixation show a clear reduction in staining.

c) Effect of deglycosylation of membrane extracts of cell line 23132 on reaction with antibody 103/51 in Western blot analysis. Extracts incubated for 16 h with deglycosylation buffer (Buffer) show no difference in staining to untreated extracts (Control). Incubation with N-glycosidase leads to a clear reduction in staining (N-glyco).

Fig. 4: Immunohistochemical staining with murine antibody 58/47-69 and 103/51 on stomach adenocarcinoma

To show identical specificity of antibody 103/51 and murine antibody 58/47-69, diffuse-type stomach adenocarcinoma was stained with haematoxilin-eosin (a), antibodies 103/51 (b) and 58/47-69 (c), and anti-cytokeratin 18 as a positive control. Identical staining in (c) and (d) indicates identical specificity (arrows = tumor cells).

Fig.5: Immunohistochemical staining of antibody 103/51 on different gastric tissues

Cryo-sections of gastric tissues were stained by HE, antibody Ki67 (to indicate proliferating cells) and antibody 103/51. (Magnification x100)

a) gastric tissue with inflammation
b) *H. pylori* induced gastritis (inlets shows magnification of marked glands.
c) Dysplasia
d) Gastric adenocarcinoma

Fig. 6: Immunohistochemical staining with antibody 103/51 on different cancerous and normal tissues

The staining of antibody 103/51 on the following tissues is shown: Carcinoma of the ampulla of Vater (a), mamma carcinoma invasive lobular (b), adenocarcinoma of the colon and no staining of normal beaker cell epithelium of the colon (c), hepatocellular carcinoma (d), glomerular and fascicular zones of the adrenal gland (e), collecting tubes of the kidney-specific staining of the Golgi apparatus (arrow) (f). Arrows in a - d indicate tumor cells, the red arrow in (c) = beaker cells, the arrow in (f) indicates Golgi apparatus (Magnification 400x, except (g) 200x).

Fig. 7: Stimulation of cell lines with antibodies 103/51 and 58/47-69 determined by colorimetric MTT-assay

a) Titration with purified antibody 103/51 shows an increase in stimulation up to 4 µg/ml. Higher concentrations do not lead to higher stimulation (c = Control, no antibody added).
b) A MTT-assay with equal concentrations (4 µg/ml) of purified antibodies 103/51 and 58/47-69 shows comparable stimulation of tumor cell 23132 after one or two days of incubation (Control 1 = chromopure human IgM, Control 2, uncorrelated mouse IgM).
c) Cell line 23132 was transiently transfected with control vector pHOOK-2 or CFR-1 antisense vector, incubated for 24 h, and tested in the MTT assay for stimulation with 4 µg/ml purified antibody 103/51 after 24 h. Untransfected cells were also incubated as control (Control, uncorrelated human IgM).

d) A MTT-assay, with equal concentrations (4 µg/ml) of antibody 103/51, on different epithelial tumor cell lines shows stimulation only on the CFR-1-positive cell line 23132 24 h after addition of antibody. CFR-1-negative cell lines Colo-699 and EPLC-272H do not show any stimulation by antibody 103/51.

Tab. 1: Reaction pattern of antibody 103/51 with different tissues

Antibody staining was scored as followed: - = no staining, + = moderate staining,
++ = intensive staining. HCC = hepatocellular carcinoma, [1] Proliferation zone, Glandular foveola, [2] Glomerular, fascicular zone (membranous staining), [3] Collecting tubes of the endoplasmatic reticulum.

Appendix A

Appendix B

Appendix S: comparison of the amino acid sequences of the CFR-1 obtained from cell line 23132 to the sequences of CFR-1 and MG160 already published.

These experimental comparisons primarily show that the CFR-1 protein obtained from cell line 23132 is not identical to the CFR-1 sequences previously known, but represents an isoform thereof. In addition to the differences in relation to the previously known and published CFR-1 and MG160, the amino acid sequence is seen as a special embodiment of the generally claimed receptor and is uniquely characterized by the first and specially identified positions.

Results

Purification and identification of antigen 103/51

Western Blot analysis was used to show that the antibody 103/51 binds to an approximately 130 kD membrane protein on stomach cancer cells. We prepurified this protein by sequential size exclusion and anion exchange chromatography (Fig. 1 a). The protein was excised from a Coomassie-stained preparative SDS-PAGE, one part was used for production of mouse monoclonal antibodies (see below), and one part was used to identify the protein using the method outlined by Shevchenko et al.

(1996, Proc. Natl. Acad. Sci. U.S.A. 93:14440-14445). After 3 h of *in-gel* digestion with trypsin, about 1% of the total digested volume was removed and subjected to high mass accuracy MALDI peptide mass mapping (saving the rest of the digest for nanoelectrospray analysis, in case MALDI MS did not lead to definitive identification). Despite the femtomole amount of the protein digest consumed for MALDI analysis, a database search matched 35 peptides to the CFR-1 sequence with a mass accuracy within 50 ppm. These peptides cover 29% of the CFR-1 sequence, thus definitively identifying the protein, which has a calculated molecular weight of approximately 134 kD (Burrus *et al.*, 1992, Mol. Cell Biol. 12:5600-5609) (Fig. 1 b).

Effect of transient transfection of cell line 23132 with CFR antisense vector on binding of antibody 103/51 and live cell staining

We investigated the effect of an antisense transfection of the stomach carcinoma cell line 23132 using immunohistochemistry and flow cytometry. For this, an 897 bp PCR-fragment of CFR, flanking the region between basepairs 802 and 1699, was cloned into the pHOOK-2 vector in an antisense direction in reference to the CMV promoter. The washed cells were transfected with the pHOOK-CFR anti-sense vector, pHOOK-lacZ, and pHOOK vector in an intermediate step. Transfection was controlled by a β-Galactosidase assay (data not shown). 48 h after transfection, cytospin preparations were prepared and stained with antibodies 103/51 and anti-cytokeratin 18 as a control (data not shown).

The immunohistochemistry showed a clear reduction of staining in cells transfected with the pHOOK-CFR antisense vector when compared to mock-transfected cells (Fig. 2 a - b). This confirmed the binding of antibody 103/51 to CFR-1. The slight cytoplasmatic staining visible in both stainings might be due to nonspecific binding often observed in staining with human IgM antibodies on acetone-fixed cells. Membrane expression and the effect of transfection were also tested by flow cytometry. (Fig. 2 g – i). The data indicates a reduction in binding of theantibody 103/51 after transfection of cells with the CFR-1 antisense vector. However, untreated cells or cells transfected with the controlvector pHOOK-2 shows a clear binding to cell line 23132, indicating expression of CFR-1 on the cell membrane.

To investigate the specific membrane distribution of the CFR-1 isoform, we performed live cell staining with cell line 23132 and some non-stomach cancer cell lines. On the cell line 23132 we found a clear staining (Fig. 2 c, d), while the human lung adenocarcinoma cell lines Colo-699 (Fig. 2 e, f) and human epidermoid lung carcinoma cell line EPLC-272H (data not shown) were clearly negative. This data show that the described CFR-1 isoform is not expressed in all cancerous cell lines, and the exclusive membrane staining of 23132 cells indicates that the CFR-1 isoform seems to have a distribution different from the one described so far for CFR-1.

Glycosidase assay

CFR-1 is a sialoglycoprotein with 5 possible N-glycosylation sites, and it has been shown shown by treatment with glycosidase F that the molecule is glycosylated at these sites (Steegmaier *et al.*, 1995, Nature 373:615-620). Since tumor-reactive antibodies often react with carbohydrate residues, we investigated whether this is the case for the antibody 103/51. Cytospin preparations of cell line 23132 were incubated for 4 h with O- and N-glycosidases, and then subjected to immunohistochemical staining with antibody 103/51. Treatment of cells with N-glycosidase led to a dramatic decrease in 103/51 staining (Fig. 3 b), while incubation with dephosphorylation buffer (Fig. 3 a) or digestion with O-glycosidase (data not shown) had no effect on binding of the antibody 103/51. This shows that the specificity of binding of the antibody 103/51 must be located in sugar residues and not in the primary protein sequence.

To further control for this effect, membrane extracts of cell line 23132 were deglycosylated for 16 h and Western blots were prepared and stained with antibody 103/51. We found a reduction in the reaction on lysates incubated with N-glycosydase when compared to the control lysates (Fig. 3 c).

Production of murine antibodies and immunohistochemical staining of paraffin section of stomach adenocarcinoma

Since commercial antibodies to CFR-1 are not available, we immunized mice with purified protein eluted from Coomassie-stained SDS-gel for production of

monoclonal antibodies to strengthen the specificity, and to further characterize CFR-1 expression. Spleen cells were immortalized by fusion with the heteromyeloma NS0. 150 clones were tested for immunohistochemical staining. Positive clones were recloned, and the clone 58/47-49 (IgM) was used for further characterization. To investigate the binding properties of the human antibody 103/51 and the murine antibody 58/47-69, we stained paraffin sections of 15 different stomach adenocarcinoma and one adenoma. Identical staining of glandular cells of the normal epithelial tissue and intensive staining of carcinoma cells was found (Fig. 4). In short, early carcinoma (n = 2) were stained by both antibodies. On intestinal-type carcinoma both antibodies stained 4 out of 5 cases, on diffuse-type carcinoma all cases (n = 4) were stained, and the intermediary-type were positive in 50 % (n = 4) with both antibodies. These results show a high expression of CFR-1 in most cases of stomach carcinoma. The investigated adenoma showed a distinct staining pattern, with positive cells only in the transition from normal to transformed cells.

Immunohistochemical staining with antibody 103/51 on gastric mucosa

To investigate the reaction pattern of antibody 103/51 on gastric mucosa in more detail, we performed immunohistochemical stainings on gastric tissue without inflammation, *H. pylori* associated chronic active gastritis, high-grade dysplasia and gastric adenocarcinoma. On non-inflammed gastric tissue no reaction was seen (Fig. 5). However, in the mucosa of a patient with *H. pylori* gastritis we found staining predominently in the basal zone of foveolar cells. The staining pattern of antibody 103/51 shows a strong correlation with the activation pattern shown by Ki67 staining (Ramires *et al.*, 1997, J. Pathol. 182:62-67). A more intensive staining of antibody 103/51 was seen in the proliferation zone of gastric dysplasia also correlating with Ki67 staining. The strongest staining was found in the proliferating zone of gastric adenocarcinoma.

Immunohistochemical staining of antibodies 103/51 and 58/47-69 on different tissues

We investigated the expression of CFR-1 in other cancerous and normal tissues by immunohistochemical staining of paraffin sections with antibodies 103/51 and 58/47-69. Out of 15 cancerous tissues (different from stomach carcinoma), antibody 103/51

showed staining in 13 cases (Fig. 6, Tab. 1a). Negative staining was observed on anaplastic cells of the lung, confirming the results from the immunohistochemical staining and MTT-assay with the cell lines Colo-699 and EPLC-272H. This data indicates an overexpression of CFR-1 and distribution to the cell membrane in malignant transformed cells. On 28 normal tissues tested, we found a restricted expression only on three intestinal organs (Tab. 1 b). Membrane staining was observed on the glandular foveola of the stomach and the glomerular and fascicular zones of the adrenal gland, while staining of the Golgi apparatus was found in the collecting tubes of the kidney (Fig. 5). This further confirms the characterization of the antigen as CFR-1, that has been described earlier by Burrus et al. (1992, Mol. Cell Biol. 12:5600-5609).

Stimulation with human and murine monoclonal antibodies

As stated in our previous publications (Vollmers *et al.*, 1994, Cancer 74:1525-1532; Hensel *et al.*, 1999, Int. J. Cancer 81:229-235), the antibody 103/51 leads to the stimulation of cell line 23132 *in vitro*. We measured this stimulation of antibody 103/51 using the mitochondrial hydroxylase assay (MTT), which is a standard assay for proliferation (Carmichael *et al.*, 1987, Cancer Res. 47:936-942). To further investigate the stimulating properties of antibody 103/51, we incubated the cell line 23132 with various concentrations of purified antibody. We found a concentration-dependent stimulation with the highest activity at 4 µg/ml (Fig. 7 a). Higher concentrations showed a slight decrease in stimulation.

To test if the murine antibody 58/47-69 has the same effects on cell growth, we performed the MTT-stimulation assay with purified antibodies in comparable amounts. As it can be seen in Fig. 7 b, both antibodies lead to the stimulation of cell line 23132 *in vitro*. This further confirms identical specificity of both antibodies.

To confirm that the stimulation of antibody 103/51 and the murine antibody 58/47-69 is mediated by binding to CFR-1, we transfected cells with control vector pHOOK-2 and CFR-1 antisense vector and tested transfected cells in the MTT-assay. As a positive control for transfection, cells were also transfected with pHOOK-2-lacZ vector followed by ß-galactosidase staining (data not shown). Since comparable

stimulation was observed in nontransfected cells and cells transfected with control vector pHOOK-2, a reduction of the stimulating effect of both antibodies by the transfection procedure can be excluded. In contrast, cells transfected with CFR-1 antisense vector clearly show a reduced stimulation (Fig. 7 c).

Finally, to demostrate that the stimulation by antibody 103/51 is not mediated by receptors other than CFR-1, we performed a MTT-stimulation assay with cell line the 23132 and compared it with the CFR-1-negative lung carcinoma cell lines Colo-699 and EPLC-272H. While the cell line 23132 is stimulated as described above, the two lung carcinoma cell lines do not show any stimulation by antibody 103/51 (Fig. 7 d), confirming the results observed in the immunohistochemistry.

Tab. 1

a) Tumor tissues

Tissue	Carcinoma-type	Antibody-Staining
Esophagus	Squamous	+
Stomach	Adeno (diffuse)	++
Stomach	Adeno (intestinal)	+
Colon	Adeno	+
Rectum	Adeno	+
Liver	Adeno (HCC)	++
Gallbladder	Adeno	+
Pancreas	Adeno (ductal)	+
Papilla of Vater	Adeno	+
Lung	Large cell anaplastic	-
Lung	Small cell	-
Lung	Adeno	++
Bronchus	Squamous epithelium	+
Mamma	Invasive (ductal)	+
Mamma	Invasive (lobular)	+

b) Normal tissues

Tissue	Cell type	Antibody-Staining
Salivary gland	Glandular	-
Stomach (non inflammated)	Glandular	-
Stomach (*H. pylori* infected)	Glandular	+[1]
Stomach (high grade dysplasia)	Glandular	++[1]
Duodenum	Glandular	-
Colon	Epithelial	-
Rectum	Glandular	-
Pancreas	Glandular	-
Liver	Glandular	-
Gallbladder	Glandular	-
Oral mucosa	Squamous epithelium	-
Anal mucosa	Squamous epithelium	-
Skin	Keratinocyte, glandular	-
Mamma	Glandular	-
Larynx	Epithelial	-
Bronchus	Epithelial	-
Lung	Glandular, alveolar	-
Thyroid gland	Glandular	-
Adenohypophysis	Glandular	-
Adrenal gland	Glandular	++[2]
Testis	Glandular	-
Ovar	Glandular	-
Prostate	Glandular	-
Urothelium	Epithelial	-
Kidney	Epithelial	++[3]
Thymus	Lymphatic	-
Spleen	Lymphatic	-
Lymph node	Lymphatic	-
Cerebral cortex	Neural	-
Peripheric neural ganglia	Neural	-

Patent Claims

What is claimed is:

1. Receptor on the surface membrane of strongly proliferating cells, particularly of gastric carcinoma, which is made up of glycoproteins,
characterized in that at least one determinant of the glycoprotein corresponds to a determinant of the CFR-1 protein; and the human antibody 103/51 and/or the murine antibody 58/47-69 (IgM) binds specifically to the glycoprotein.

2. Receptor according to Claim 1,
characterized in that the specific binding site on the glycoprotein is a carbohydrate residue (= sugar residue).

3. Receptor according to Claim 1,
characterized in that the primary amino acid sequence of the glycoprotein corresponds at least 80% to that of CFR-1 (is homologous).

4. Receptor according to Claim 1,
characterized in that the determinants of the glycoprotein have the amino acid sequence reproduced in Appendix S, cell line 23132.

5. Receptor according to one of Claims 1 to 4,
characterized by a molecular mass of approximately 130 kD.

6. Use of the receptor according to one of the preceding claims,
characterized in that the receptor is administered *in vivo* to induce the formation of antibodies.

7. Use of the receptor according to one of the preceding claims for the treatment of tumors,
characterized in that the receptor is administered before (for prophylaxis) or with the outbreak of the illness (for therapy).

8. Use of the receptor according to one of the preceding claims for the treatment of the following tumors: esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lung, bronchi, breast, cervix, prostate, cardiac, Barrett's, ovary, and/or uterus.

9. Use of the receptor according to one of the preceding claims for the treatment of the following tumor precursors:

of the stomach:
- dysplasia of the gastric mucosa
- intestinal metaplasia of the stomach
- *Helicobacter pylori*-associated gastritis
- tubular and tubulovillous adenoma of the stomach

of the large intestine:
- tubular adenoma of the colon
- villous adenoma of the colon
- dysplasia in ulcerative colitis

in the esophagus:
- Barrett's dysplasia of the esophagus
- Barrett's metaplasia of the esophagus

of the cervix:
- cervical intraepithelial neoplasia I
- cervical intraepithelial neoplasia II
- cervical intraepithelial neoplasia III

of the lungs:
- squamous epithelial metaplasia of the bronchus
- squamous epithelial dysplasia of the bronchus.

10. Use of the receptor according to one of the preceding claims for diagnostic purposes,
characterized in that evidence for the existence, the localization, and/or the quantity of the corresponding antibodies and/or receptors is obtained via the ability of antibodies to bind to the receptor.

11. Use according to Claim 10,
characterized in that the antibodies are tumor antibodies.

12. Use according to Claim 10,
characterized in that the receptor is a tumor marker.

13. Method of extracting the receptor according to one of the preceding claims, **characterized by** the following steps:
a) preparation of membrane proteins from cells of the human adenocarcinoma cell line 23132
b) performing size exclusion chromatography and
c) anion exchange chromatography and
d) finally extraction through preparative SDS-PAGE.

14. Murine mouse antibody 58/47-69 for use in one of the preceding claims and a structure which is characterized by the following features:
the variable region of the heavy chain is homologous to IGHV 1S 125* 01 according to Appendix A, the D segment being homologous to IGHD-ST 4*01 and the J segment being homologous to IGHJ4*01, and the variable region of the light chain has a structure according to Appendix B, which is homologous to IGKV-17*01, the J segment being homologous to IGKJ2*01.

15. Method of producing an antitumor agent using receptors according to one of the preceding claims,

characterized in that a compound with potential antitumor activity is tested for its ability to specifically bind to receptors according to one of the preceding claims and, in the event of a positive result, this compound is formulated for pharmaceutical administration and provided with typical additives for this purpose.

16. Method of producing an antitumor agent using receptors according to Claim 15,

characterized in that the compounds are human antibodies and/or mouse antibodies and/or humanized mouse antibodies and/or Fab and $F(ab)_2$ and Fab' fragments and/or single strand antibodies and/or tetrameric and/or dimeric antibody forms and/or bispecific antibodies.

Article 34 Patent Claims

1. Receptor on the surface membrane of strongly proliferating cells, particularly of gastric carcinoma, which is made up of glycoproteins that specifically bind the human antibody 103/51, and which exhibits a molecular weight of approximately 130 kD, **characterized in that** at least one determinant of the glycoprotein corresponds to a determinant of the CFR-1 protein; the determinant of the glycoprotein exhibits the amino acid sequence shown in SEQ ID NO:6, the specific binding site on the glycoprotein is a carbohydrate residue (= sugar residue), and the murine antibody 58/47-69 (IgM) binds specifically to the glycoprotein.

2. Receptor according to Claim 1,
characterized in that the primary amino acid sequence of the glycoprotein corresponds at least 80% to that of CFR-1 (is homologous).

3. Use of the receptor according to one of the preceding claims for the manufacture of a medicament that is administered to induce the *in vivo* formation of antibodies.

4. Use of the receptor for the manufacture of a medicament for the treatment of tumors according to one of the preceding claims,
characterized in that the receptor is administered before (for prophylaxis) or with the outbreak of the illness (for therapy).

5. Use of the receptor for the manufacture of a medicament according to one of the preceding claims for the treatment of the following tumors: esophagus, stomach, intestine, rectum, liver, gallbladder, pancreas, lung, bronchi, breast, cervix, prostate, cardiac, Barrett's, ovary, and/or uterus.

6. Use of the receptor for the manufacture of a medicament according to one of the preceding claims for the treatment of the following tumor precursors:

of the stomach:

- dysplasia of the gastric mucosa
- intestinal metaplasia of the stomach
- *Helicobacter pylori*-associated gastritis
- tubular and tubulovillous adenoma of the stomach

of the large intestine:
- tubular adenoma of the colon
- villous adenoma of the colon
- dysplasia in ulcerative colitis

in the esophagus:
- Barrett's dysplasia of the esophagus
- Barrett's metaplasia of the esophagus

of the cervix:
- cervical intraepithelial neoplasia I
- cervical intraepithelial neoplasia II
- cervical intraepithelial neoplasia III

of the lung:
- squamous epithelial metaplasia of the bronchus
- squamous epithelial dysplasia of the bronchus.

7. Diagnostic agent according to one of claims 1 and 2, **characterized in that** the receptor specifically binds antibodies.

8. Diagnostic agent according to Claim 7, **characterized in that** the antibodies are tumor antibodies.

9. Diagnostic agent according to Claim 7, **characterized in that** the receptor is a tumor marker.

10. Method of extracting the receptor according to one of the preceding claims, **characterized by** the following steps:

a) preparation of membrane proteins from cells of the human adenocarcinoma cell line 23132

b) performing size exclusion chromatography and

c) anion exchange chromatography and

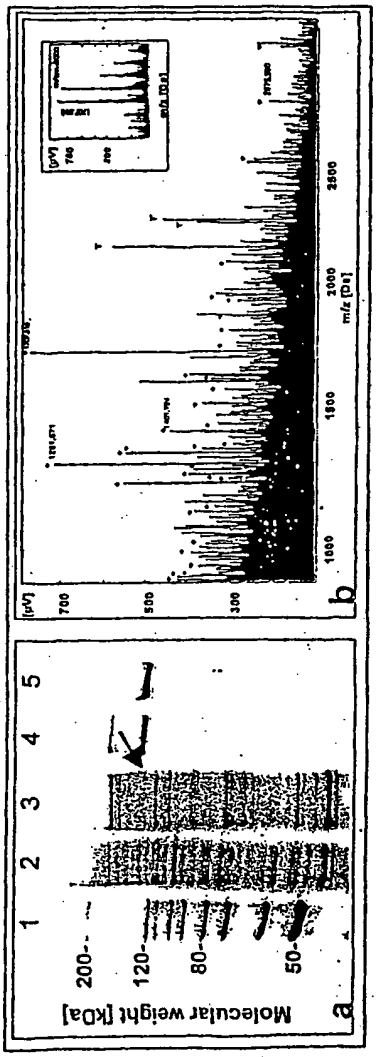
d) finally extraction through preparative SDS-PAGE.

11. Murine mouse antibody 58/47-69 for use in one of the preceding claims and a structure which is characterized by the following features:
the variable region of the heavy chain is homologous to IGHV 1S 125* 01 according to SEQ ID NO:1, the D segment being homologous to IGHD-ST 4*01 and the J segment being homologous to IGHJ4*01, and the variable region of the light chain has a structure according to SEQ ID NO:3, which is homologous to IGKV-17*01, the J segment being homologous to IGKJ2*01.

12. Method of producing an antitumor agent using receptors according to one of the preceding claims,
characterized in that a compound with potential antitumor activity is tested for its ability to specifically bind to receptors according to one of the preceding claims and, in the event of a positive result, this compound is formulated for pharmaceutical administration and provided with typical additives for this purpose.

13. Method of producing an antitumor agent using receptors according to Claim 12, **characterized in that** the compounds are human antibodies and/or mouse antibodies and/or humanized mouse antibodies and/or Fab and $F(ab)_2$ and Fab' fragments and/or single strand antibodies and/or tetrameric and/or dimeric antibody forms and/or bispecific antibodies.

Fig. 1



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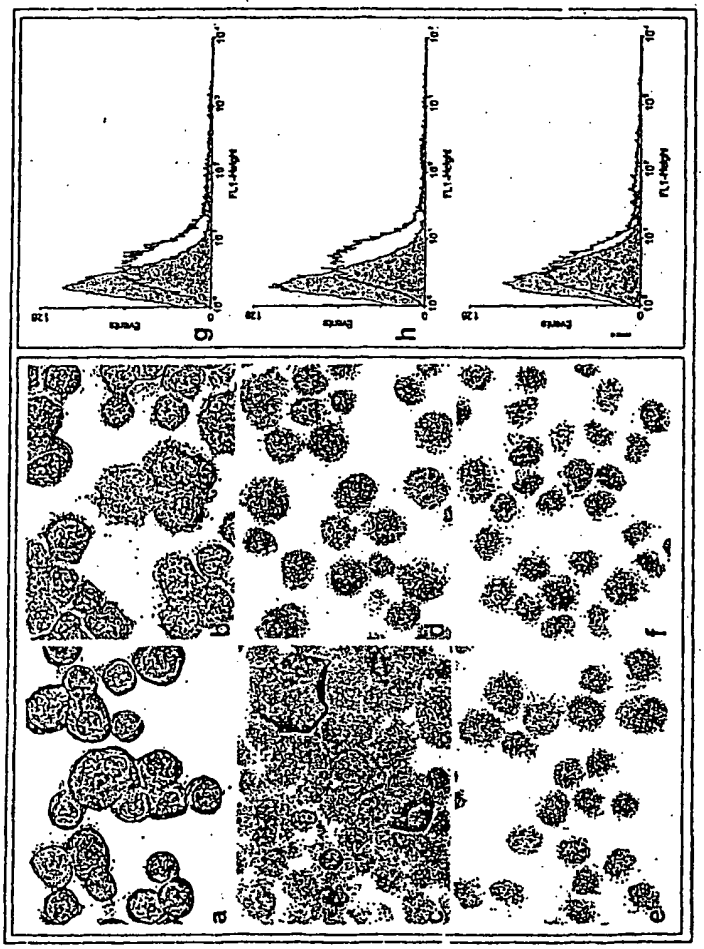
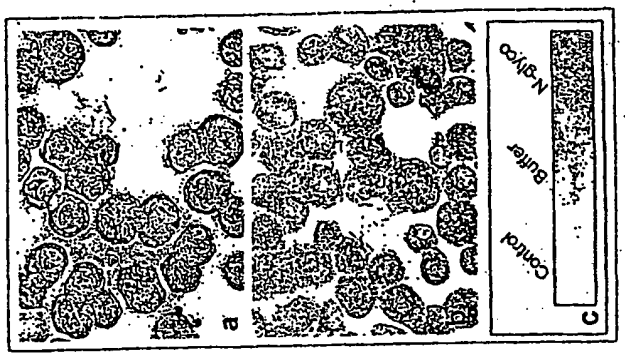


Fig. 3



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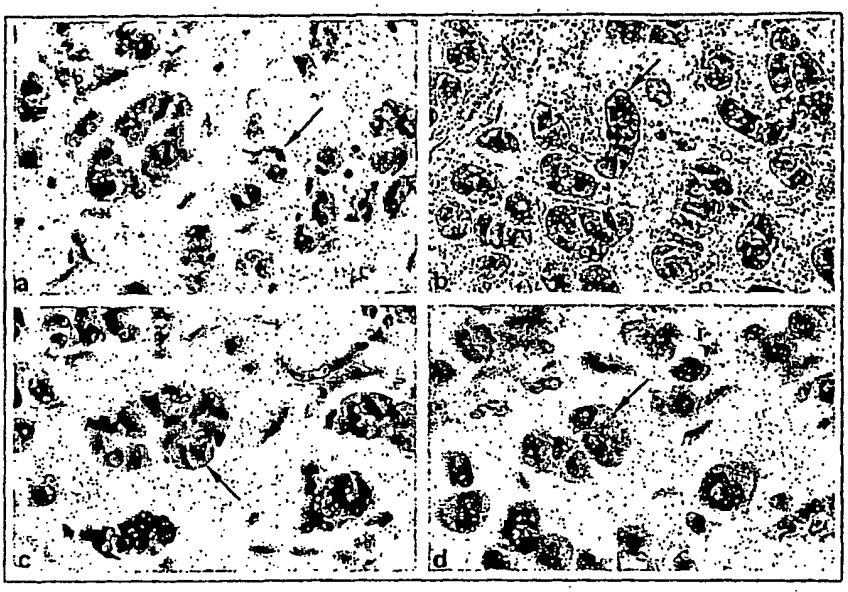

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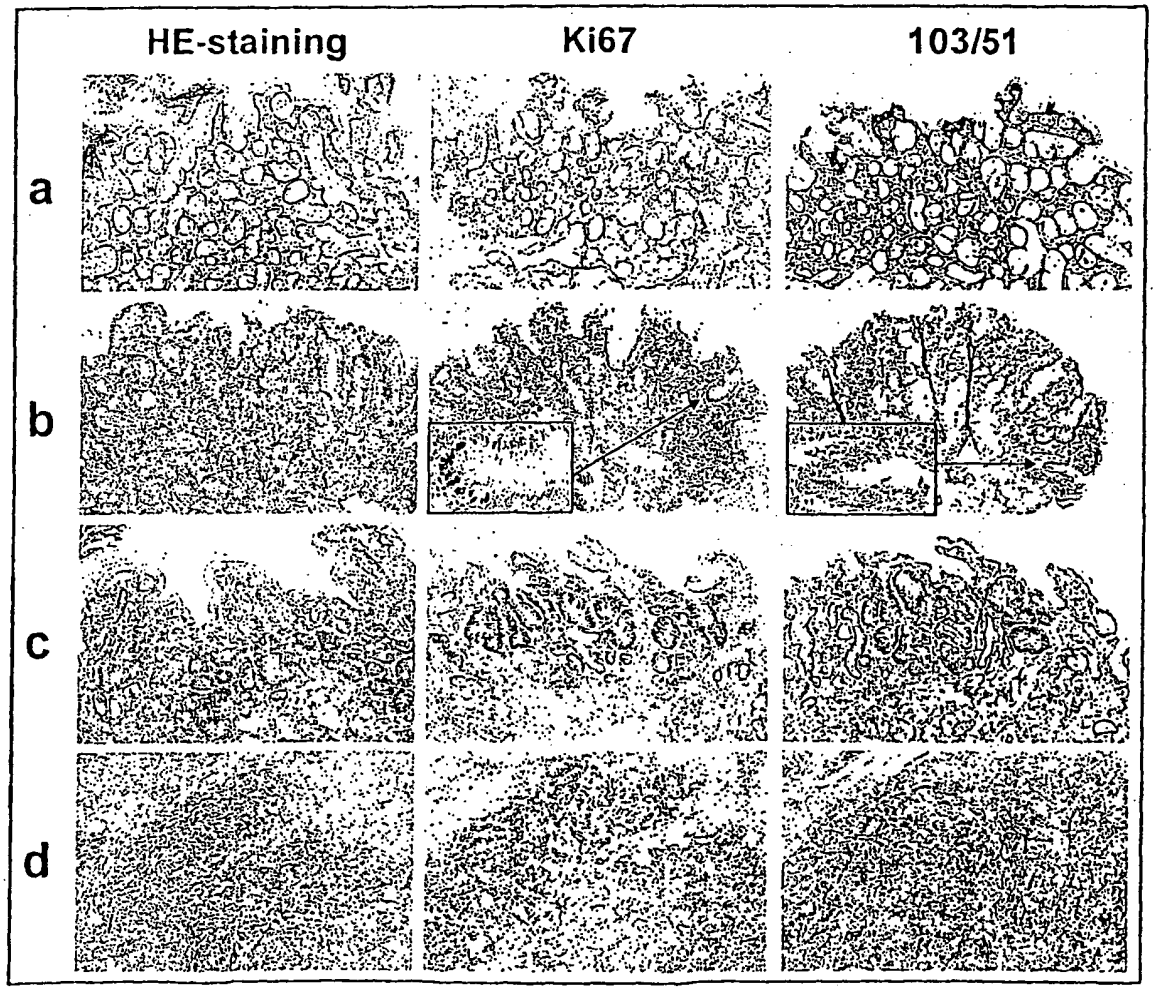


Fig. 6

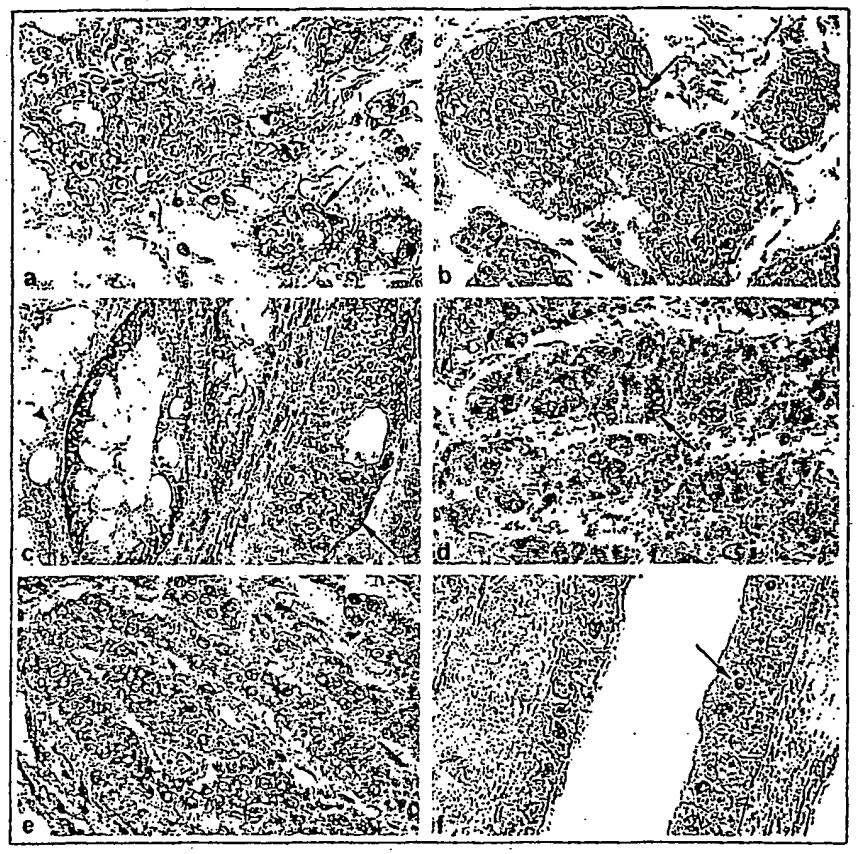
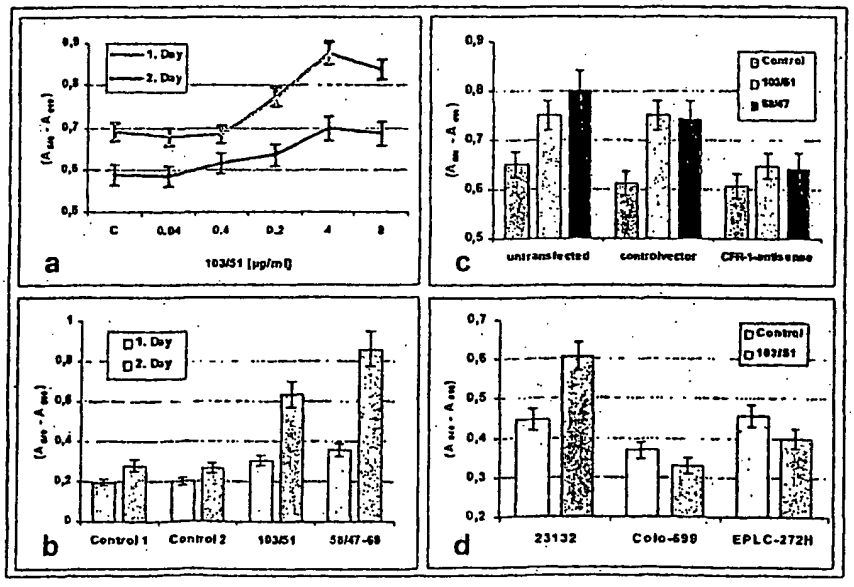

a
b
c
d
e
f

Fig. 7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2. Day
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b
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Control 2
103/51
58/47-69
c
Control
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58/47
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controlvector
CFR-1-antisense
d
Control
103/51
23132
Colo-699
EPLC-272H

Appendix A

<110> Prof. Dr. Müller-Hermelink, Hans Konrad
Prof. Dr. Vollmers, Heinz
Dr. Hensel, Frank

<112> Receptor, its use, and mouse antibody

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Arg
 1               5                  10                  15
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act gga cag ggc ctt gag tgg att gga gag att tat cct gga agt ggt aat act tac
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Thr Gly Gln Gly Leu Glu Trp Ile Gly Glu Ile Tyr Pro Gly Ser Gly Asn Thr Tyr
Tyr
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Gly
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65
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<110> Prof. Dr. Müller-Hermelink, Hans Konrad
Prof. Dr. Vollmers, Heinz
Dr. Hensel, Frank

<112> Receptor, its use, and mouse antibody

<141> 03/09/02

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Gln
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20

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Phe
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Thr
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100

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315
Lys Leu Glu Ile Lys
                105
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<110> Prof. Dr. Müller-Hermelink, Hans Konrad
Prof. Dr. Vollmers, Heinz
Dr. Hensel, Frank

<112> Receptor, its use, and mouse antibody

<141> 03/09/02

<211> 3114

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Trp Asn Tyr
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160

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TGC AAA TCT
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Cys Lys Ser
              165                 170                 175
180

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ATG GTT TCC
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Met Val Ser
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200

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ATT ACC AAG
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Ile Thr Lys
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220
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Met Thr Ala Ile Ile Phe Ser Asp Tyr Arg Leu Ile Cys Gly Phe Met Asp Asp Cys Lys
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245 250 255 260

CAA GGT GAG GTG GTA TCA TGC TTG GAG AAA GGC CTG GTG AAA GAA GCA GAA GAA AGA GAA
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265 270 275 280

CCC AAG ATT CAA GTT TCT GAA CTC TGC AAG AAA GCC ATT CTC CGG GTG GCT GAG CTG TCA
Pro Lys Ile Gln Val Ser Glu Leu Cys Lys Lys Ala Ile Leu Arg Val Ala Glu Leu Ser
285 290 295 300

TCG GAT GAC TTT CAC TTA GAC CGG CAT TTA TAT TTT GCT TGC CGA GAT GAT CGG GAG CGT
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325 330 335 340

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CAC TGT CTG ATG AAA GTA GTT CGA GGG GAG AAG GGG AAC CTT GGA ATG AAC TGC CAG CAG
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705 710 715 720

CAG AAC AAA CAC CAG AAG GAC ATG AAC GAG AAG TGT GCC ATC GGA GTT ACC CAC TTC CAG
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CTG GTG CAG ATG AAG GAT TTT CGG TTT TCT TAC AAG TTT AAA ATG GCC TGC AAG GAG GAC
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745 750 755 760

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765 770 775 780

ACC GTG CGC AAT GAC ACT CTG CAG GAA GCC AAG GAG CAC AGG GTG TCC CTG AAG TGC CGC
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785 790 795 800

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805 810 815 820

TAC GAA GCC TGC AAG AGT GAC ATC AAA AAC TTC TGT TCC GCT GTG CAA TAT GGC AAC GCT
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825 830 835 840

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Ala Ile
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Arg Val
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Ser Pro
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Ile Gly
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INTERNATIONAL SEARCH REPORT

Int ɔnal Application No
PCT/DE 02/02699

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/705 A61K38/16 C07K16/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
SEQUENCE SEARCH, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HENSEL FRANK ET AL: "A new variant of cystein-rich FGF receptor (CFR-1) specifically expressed on tumor cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, vol. 41, March 2000 (2000-03), page 698 XP001154022 91st Annual Meeting of the American Association for Cancer Research.;San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X the whole document --- -/--	1-15

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 August 2003	22/08/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Herrmann, K

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	MOURELATOS ZISSIMOS ET AL: "Cloning and sequence analysis of the human MG160, a fibroblast growth factor and E-selectin binding membrane sialoglycoprotein of the Golgi apparatus." DNA AND CELL BIOLOGY, vol. 15, no. 12, 1996, pages 1121-1128, XP001154021 ISSN: 1044-5498 99,7% identisch mit SEQ ID NO:6 in einem Überlappungsbereich von 1037 Aminosäuren the whole document ---	1-3,5-15
X	HENSEL FRANK ET AL: "Mitogenic autoantibodies in Helicobacter pylori-associated stomach cancerogenesis." INTERNATIONAL JOURNAL OF CANCER, vol. 81, no. 2, 12 April 1999 (1999-04-12), pages 229-235, XP002250371 ISSN: 0020-7136 cited in the application page 233, left-hand column, line UNTEN; figure 2 ---	1-15
A	VOLLMERS H PETER ET AL: "Human monoclonal antibodies from stomach carcinoma patients react with Helicobacter pylori and stimulate stomach cancer cells in vitro." CANCER (PHILADELPHIA), vol. 74, no. 5, 1994, pages 1525-1532, XP009015348 ISSN: 0008-543X cited in the application the whole document ---	1-15
A	VOLLMERS H PETER ET AL: "Adjuvant therapy for gastric adenocarcinoma with the apoptosis-inducing human monoclonal antibody SC-1: First clinical and histopathological results." ONCOLOGY REPORTS, vol. 5, no. 3, May 1998 (1998-05), pages 549-552, XP009015315 ISSN: 1021-335X cited in the application the whole document --- -/--	1-15

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	HENSEL FRANK ET AL: "A novel proliferation-associated variant of CFR-1 defined by a human monoclonal antibody." LABORATORY INVESTIGATION, vol. 81, no. 8, August 2001 (2001-08), pages 1097-1108, XP002250372 ISSN: 0023-6837 the whole document	1-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/DE 02/02699

BOX I. I.

Although Claims 6-12 relate to a method for treatment of the human or animal body or to a diagnostic method practiced on the human or animal body, the search was carried out on the basis of the alleged effects of the compound or composition.

BOX I.2

Claim No: 16

Claim 16 lacks the requisite clarity (PCT Article 6) to such an extent that it is impossible to carry out a meaningful search (PCT Article 17(1)(a)(ii)). Claim 16 relates to "receptors according to Claim 15". Claim 15, however, is a method claim.

The applicant is advised that claims or parts of claims relating to inventions in respect of which no international search report has been established normally cannot be the subject of an international preliminary examination (PCT Rule 66.1(e)). In its capacity as International Preliminary Examining Authority the EPO generally will not carry out a preliminary examination for subjects that have not been searched. This also applies to cases where the claims were amended after receipt of the international search report (PCT Article 19) or where the applicant submits new claims in the course of the procedure under PCT Chapter II.

INTERNATIONALER RECHERCHENBERICHT

Inte, inales Aktenzeichen
PCT/DE 02/02699

A. KLASSIFIZIERUNG DES ANMELDUNGSGEGENSTANDES
IPK 7 C07K14/705 A61K38/16 C07K16/28

Nach der Internationalen Patentklassifikation (IPK) oder nach der nationalen Klassifikation und der IPK

B. RECHERCHIERTE GEBIETE

Recherchierter Mindestprüfstoff (Klassifikationssystem und Klassifikationssymbole)
IPK 7 C07K A61K

Recherchierte aber nicht zum Mindestprüfstoff gehörende Veröffentlichungen, soweit diese unter die recherchierten Gebiete fallen

Während der internationalen Recherche konsultierte elektronische Datenbank (Name der Datenbank und evtl. verwendete Suchbegriffe)
SEQUENCE SEARCH, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ, EMBASE, SCISEARCH

C. ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	HENSEL FRANK ET AL: "A new variant of cystein-rich FGF receptor (CFR-1) specifically expressed on tumor cells." PROCEEDINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNUAL, Bd. 41, März 2000 (2000-03), Seite 698 XP001154022 91st Annual Meeting of the American Association for Cancer Research.;San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X das ganze Dokument --- -/--	1-15

[X] Weitere Veröffentlichungen sind der Fortsetzung von Feld C zu entnehmen

[ ] Siehe Anhang Patentfamilie

* Besondere Kategorien von angegebenen Veröffentlichungen :
'A' Veröffentlichung, die den allgemeinen Stand der Technik definiert, aber nicht als besonders bedeutsam anzusehen ist
'E' älteres Dokument, das jedoch erst am oder nach dem internationalen Anmeldedatum veröffentlicht worden ist
'L' Veröffentlichung, die geeignet ist, einen Prioritätsanspruch zweifelhaft erscheinen zu lassen, oder durch die das Veröffentlichungsdatum einer anderen im Recherchenbericht genannten Veröffentlichung belegt werden soll oder die aus einem anderen besonderen Grund angegeben ist (wie ausgeführt)
'O' Veröffentlichung, die sich auf eine mündliche Offenbarung, eine Benutzung, eine Ausstellung oder andere Maßnahmen bezieht
'P' Veröffentlichung, die vor dem internationalen Anmeldedatum, aber nach dem beanspruchten Prioritätsdatum veröffentlicht worden ist
'T' Spätere Veröffentlichung, die nach dem internationalen Anmeldedatum oder dem Prioritätsdatum veröffentlicht worden ist und mit der Anmeldung nicht kollidiert, sondern nur zum Verständnis des der Erfindung zugrundeliegenden Prinzips oder der ihr zugrundeliegenden Theorie angegeben ist
'X' Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann allein aufgrund dieser Veröffentlichung nicht als neu oder auf erfinderischer Tätigkeit beruhend betrachtet werden
'Y' Veröffentlichung von besonderer Bedeutung; die beanspruchte Erfindung kann nicht als auf erfinderischer Tätigkeit beruhend betrachtet werden, wenn die Veröffentlichung mit einer oder mehreren anderen Veröffentlichungen dieser Kategorie in Verbindung gebracht wird und diese Verbindung für einen Fachmann naheliegend ist
'&' Veröffentlichung, die Mitglied derselben Patentfamilie ist

Datum des Abschlusses der internationalen Recherche	Absendedatum des internationalen Recherchenberichts
6. August 2003	22/08/2003

Name und Postanschrift der Internationalen Recherchenbehörde	Bevollmächtigter Bediensteter
Europäisches Patentamt, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Herrmann, K

Formblatt PCT/ISA/210 (Blatt 2) (Juli 1992)

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie*	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
X	MOURELATOS ZISSIMOS ET AL: "Cloning and sequence analysis of the human MG160, a fibroblast growth factor and E-selectin binding membrane sialoglycoprotein of the Golgi apparatus." DNA AND CELL BIOLOGY, Bd. 15, Nr. 12, 1996, Seiten 1121-1128, XP001154021 ISSN: 1044-5498 99,7% identisch mit SEQ ID NO:6 in einem Überlappungsbereich von 1037 Aminosäuren das ganze Dokument ---	1-3,5-15
X	HENSEL FRANK ET AL: "Mitogenic autoantibodies in Helicobacter pylori-associated stomach cancerogenesis." INTERNATIONAL JOURNAL OF CANCER, Bd. 81, Nr. 2, 12. April 1999 (1999-04-12), Seiten 229-235, XP002250371 ISSN: 0020-7136 in der Anmeldung erwähnt Seite 233, linke Spalte, Zeile UNTEN; Abbildung 2 ---	1-15
A	VOLLMERS H PETER ET AL: "Human monoclonal antibodies from stomach carcinoma patients react with Helicobacter pylori and stimulate stomach cancer cells in vitro." CANCER (PHILADELPHIA), Bd. 74, Nr. 5, 1994, Seiten 1525-1532, XP009015348 ISSN: 0008-543X in der Anmeldung erwähnt das ganze Dokument ---	1-15
A	VOLLMERS H PETER ET AL: "Adjuvant therapy for gastric adenocarcinoma with the apoptosis-inducing human monoclonal antibody SC-1: First clinical and histopathological results." ONCOLOGY REPORTS, Bd. 5, Nr. 3, Mai 1998 (1998-05), Seiten 549-552, XP009015315 ISSN: 1021-335X in der Anmeldung erwähnt das ganze Dokument --- -/--	1-15

INTERNATIONALER RECHERCHENBERICHT

Internales Aktenzeichen
PCT/DE 02/02699

C.(Fortsetzung) ALS WESENTLICH ANGESEHENE UNTERLAGEN

Kategorie°	Bezeichnung der Veröffentlichung, soweit erforderlich unter Angabe der in Betracht kommenden Teile	Betr. Anspruch Nr.
P,X	HENSEL FRANK ET AL: "A novel proliferation-associated variant of CFR-1 defined by a human monoclonal antibody." LABORATORY INVESTIGATION, Bd. 81, Nr. 8, August 2001 (2001-08), Seiten 1097-1108, XP002250372 ISSN: 0023-6837 das ganze Dokument	1-15

INTERNATIONALER RECHERCHENBERICHT

Internationales Aktenzeichen
PCT/DE 02/02699

Feld I Bemerkungen zu den Ansprüchen, die sich als nicht recherchierbar erwiesen haben (Fortsetzung von Punkt 2 auf Blatt 1)

Gemäß Artikel 17(2)a) wurde aus folgenden Gründen für bestimmte Ansprüche kein Recherchenbericht erstellt:

1. [X] Ansprüche Nr.
weil sie sich auf Gegenstände beziehen, zu deren Recherche die Behörde nicht verpflichtet ist, nämlich

siehe Zusatzblatt WEITERE ANGABEN PCT/ISA/210

2. [X] Ansprüche Nr. 16
weil sie sich auf Teile der internationalen Anmeldung beziehen, die den vorgeschriebenen Anforderungen so wenig entsprechen, daß eine sinnvolle internationale Recherche nicht durchgeführt werden kann, nämlich

siehe Zusatzblatt WEITERE ANGABEN PCT/ISA/210

3. [ ] Ansprüche Nr.
weil es sich dabei um abhängige Ansprüche handelt, die nicht entsprechend Satz 2 und 3 der Regel 6.4 a) abgefaßt sind.

Feld II Bemerkungen bei mangelnder Einheitlichkeit der Erfindung (Fortsetzung von Punkt 3 auf Blatt 1)

Die internationale Recherchenbehörde hat festgestellt, daß diese internationale Anmeldung mehrere Erfindungen enthält:

1. [ ] Da der Anmelder alle erforderlichen zusätzlichen Recherchengebühren rechtzeitig entrichtet hat, erstreckt sich dieser internationale Recherchenbericht auf alle recherchierbaren Ansprüche.

2. [ ] Da für alle recherchierbaren Ansprüche die Recherche ohne einen Arbeitsaufwand durchgeführt werden konnte, der eine zusätzliche Recherchengebühr gerechtfertigt hätte, hat die Behörde nicht zur Zahlung einer solchen Gebühr aufgefordert.

3. [ ] Da der Anmelder nur einige der erforderlichen zusätzlichen Recherchengebühren rechtzeitig entrichtet hat, erstreckt sich dieser internationale Recherchenbericht nur auf die Ansprüche, für die Gebühren entrichtet worden sind, nämlich auf die Ansprüche Nr.

4. [ ] Der Anmelder hat die erforderlichen zusätzlichen Recherchengebühren nicht rechtzeitig entrichtet. Der internationale Recherchenbericht beschränkt sich daher auf die in den Ansprüchen zuerst erwähnte Erfindung; diese ist in folgenden Ansprüchen erfaßt:

Bemerkungen hinsichtlich eines Widerspruchs

[ ] Die zusätzlichen Gebühren wurden vom Anmelder unter Widerspruch gezahlt.

[ ] Die Zahlung zusätzlicher Recherchengebühren erfolgte ohne Widerspruch.

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Fortsetzung von Feld I.1

Obwohl die Ansprüche 6-12 sich auf ein Verfahren zur Behandlung des menschlichen/tierischen Körpers oder ein Diagnostizierverfahren, das am menschlichen/tierischen Körper vorgenommen wird, beziehen, wurde die Recherche durchgeführt und gründete sich auf die angeführten Wirkungen der Verbindung/Zusammensetzung.

Fortsetzung von Feld I.2

Ansprüche Nr.: 16

Anspruch 16 entspricht den vorgeschriebenen Anforderungen bezüglich Klarheit (Art. 6 PCT) so wenig, daß eine eine sinnvolle Recherche nicht durchgeführt werden kann (Art. 17(2)(a)(ii) PCT). Anspruch 16 bezieht sich auf "Rezeptoren nach Anspruch 15". Anspruch 15 ist jedoch ein Verfahrensanspruch.

Der Anmelder wird darauf hingewiesen, daß Patentansprüche, oder Teile von Patentansprüchen, auf Erfindungen, für die kein internationaler Recherchenbericht erstellt wurde, normalerweise nicht Gegenstand einer internationalen vorläufigen Prüfung sein können (Regel 66.1(e) PCT). In seiner Eigenschaft als mit der internationalen vorläufigen Prüfung beauftragte Behörde wird das EPA also in der Regel keine vorläufige Prüfung für Gegenstände durchführen, zu denen keine Recherche vorliegt. Dies gilt auch für den Fall, daß die Patentansprüche nach Erhalt des internationalen Recherchenberichtes geändert wurden (Art. 19 PCT), oder für den Fall, daß der Anmelder im Zuge des Verfahrens gemäß Kapitel II PCT neue Patentansprüche vorlegt.